The Effect of Mechanical Stimulation on the Keratinization of Sulcular Epithelium*

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THIS STUDY was designed to evaluate the effect of mechanical stimulation on the keratinization of the sulcular epithelium in four adult Rhesus monkeys. Each animal received a thorough prophylaxis. One week later, each monkey received one of the following modalities of plaque control: (a) daily intravenous tetracycline and rubber cup prophylaxis, (b) daily rubber cup prophylaxis, (c) daily intravenous tetracycline injections; (d) no treatment, as a control. After sacrifice and tissue processing the histologic sections were evaluated for the presence of sulcular keratinization. The keratin width and length were measured, and an Inflammatory Index determined. It was found that all treatment modalities reduced inflammation significantly, when compared to the control. No differences among the three procedures tested were found. Although all permitted keratinization to develop, sulcular keratinization was significantly increased when daily prophylaxes were performed. It was concluded that mechanical stimulation of the sulcular epithelium, seemingly plays a role in promoting its keratinization.

Previous studies have shown the keratinizing possibility of the sulcular epithelium *in situ*, when different regimes of antimicrobial treatments were applied in monkeys.[1, 2] These treatments have all combined the use of antimicrobials, such as systemic antibiotics and local chlorhexidine applications, with the daily mechanical removal of dental plaque by means of a rubber cup prophylaxis. All combined regimes tested allowed the sulcular epithelium to keratinize. However, the question remains as to whether the keratinization is mainly due to the almost complete elimination of the dental plaque or to the mechanical stimulation produced by the daily rubber cup prophylaxes on the sulcular epithelium.

The purpose of this study was to try to elucidate the specific effect of the mechanical stimulation produced by the prophylaxes on the keratinization of the sulcular epithelium.

MATERIALS AND METHODS

Four adult male Rhesus monkeys (*Macaca mulatta*) were used. All animals had full complements of permanent teeth with moderate supra- and subgingival calculus and generalized moderate marginal gingivitis. One week prior to the experiment all teeth were scaled and polished.

On day 0, all monkeys were placed on specific plaque control regimes, as follows: Monkey 1 received daily intravenous (IV) tetracycline hydrochloride (Achromycin®) at the maximum safe dosage of 20 mg/kg body weight and daily rubber cup prophylaxes throughout the 1 month investigation period. Monkey 2 received daily prophylaxes during the whole month investigation period. Monkey 3 received daily injections of tetracycline, using the same route and dosage as that given to Monkey 1, for 1 month. During this time this monkey received no prophylaxes. Monkey 4 served as a control and received no additional treatment.

During the experimental period, for any treatment, all monkeys were premedicated with ketamine hydrochloride, 25 mg/kg body weight, to insure cooperation. The prophylaxes consisted of a rubber cup polish with prophylaxis paste (Nupro®). They were performed during the 5 weekdays only, whereas the tetracycline administration was not interrupted during the weekends.

After sacrifice, the animals were decapitated and the jaws with the teeth were fixed in 10% neutral buffered formalin for 4 weeks. Then they were divided into small specimens and decalcified in 10% formic acid. Following decalcification, the specimens were washed in running water overnight, embedded in paraffin and sectioned

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buccolingually at 6 μ. The resulting sections were stained with either Ehrlich's acid hematoxylin and eosin, Mallory connective tissue stain as modified by Ayoub and Shklar,[3] or Rhodamine B.

Histologic Evaluation. A total of 200 randomly selected sections per monkey (50 per quadrant) stained with Mallory or Rhodamine B were evaluated for the presence or absence of keratin in the sulcular area. When present, the length and width of the keratin layer was measured using a Filar Micrometer Eyepiece (Bausch and Lomb).[2] In addition, the widths of the keratin layer in the oral gingival epithelium of the same specimens were also measured.

The corresponding sections stained with hematoxylin and eosin were used to determine an Inflammatory Index in the connective tissue underlying the sulcular epithelium, by counting the number of inflammatory cells within a microscopic field.[2] Both buccal and lingual areas were included for all the evaluations.

The data obtained were statistically analyzed in MIDAS (Michigan Interactive Data Analysis System) using the analysis of variance, Scheffe's method of multiple comparisons and the paired *t* test.

RESULTS

Each antiplaque regime tested was effective in producing keratinization of the sulcular epithelium (Fig. 1–3). As expected, no keratinization was found in the sulcular areas of the control monkey (Fig. 4).

Individual means for all parameters analyzed, Inflammatory Index, keratin width and keratin length, were determined for the buccal and lingual areas in the experimental monkeys. An Inflammatory Index was also determined in the control monkey. Since buccal and lingual means were found to be no different, combined means were obtained and used for further analysis.

Table 1 presents the mean values obtained with the different combinations of treatment as well as in the control animal. The equality of the mean values of the variables in each of the relevant groups was tested by the analysis of variance and significant differences ($P < 0.001$) were found in each instance. Scheffe's method of multiple comparisons was then used to test each of the pairwise differences at the 5% level of significance. For the Inflammatory Index, the control group was significantly different from each of the treatment groups, but

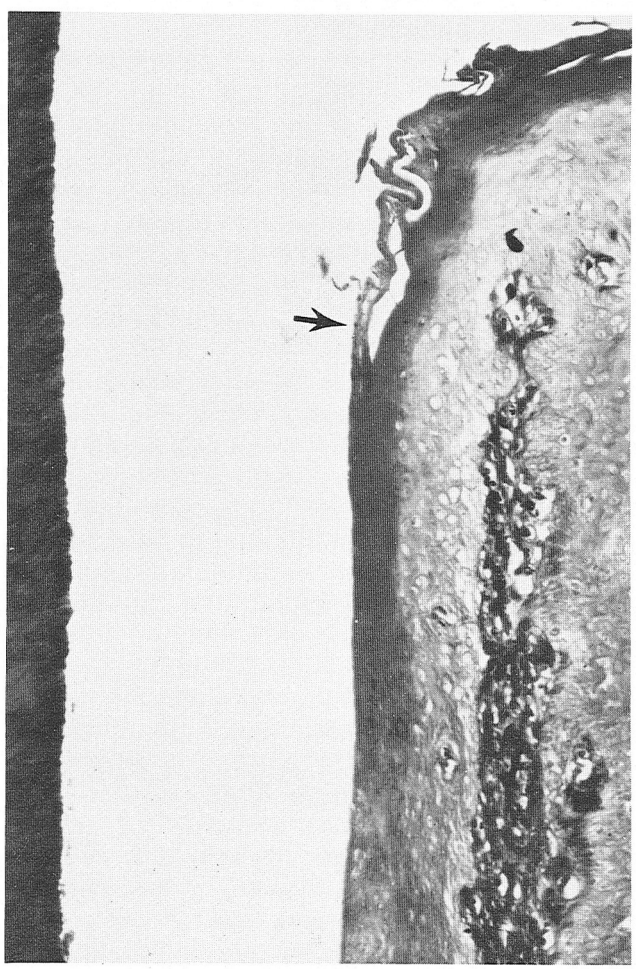

Figure 1. *Specimen from monkey treated with IV tetracycline and daily rubber cup prophylaxes. A definite band of keratin is present lining the sulcular area (Mallory stain, magnification × 100).*

Figure 2. *Specimen from monkey receiving daily prophylaxes. Observe the development of sulcular keratinization and minimal inflammation within the connective tissue (Mallory stain, magnification × 100).*

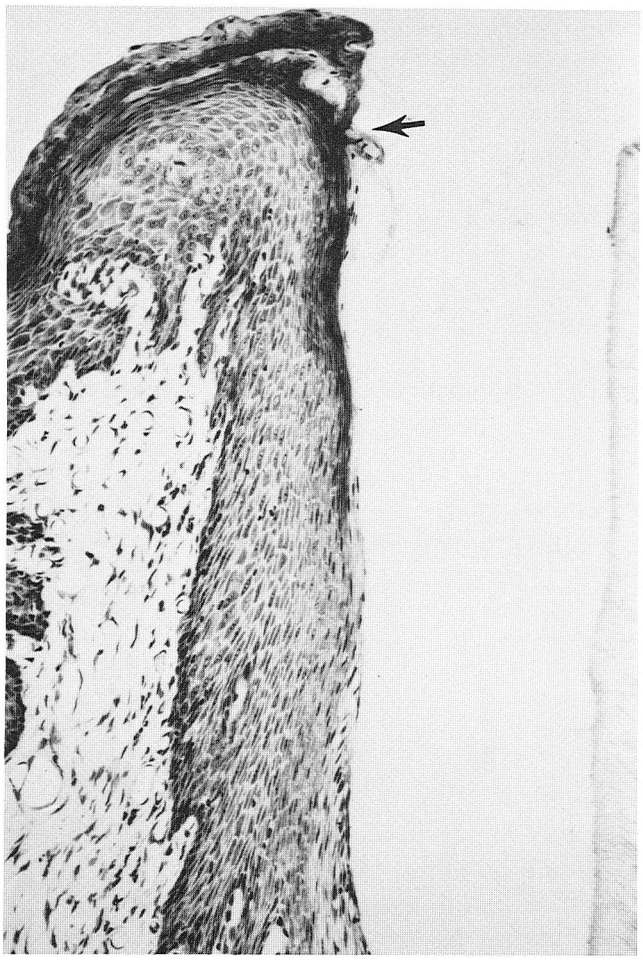

Figure 3. *Specimen from monkey treated with systemic IV tetracycline only. Minimal sulcular keratinization is present. Note the almost absence of inflammation within the tissues (Mallory stain, magnification × 100).*

Figure 4. *Specimen from the control monkey. Keratinization stops at the gingival margin (Mallory stain, magnification × 100).*

there were no significant differences between the treatment groups. Keratin length was significantly less in group 3, but groups 1 and 2 were not significantly different. Pairwise comparisons proved similar results for keratin width, with group 3 showing statistically significant deviation from group 1 and 2.

Table 2 presents the comparison between the keratin width obtained in the sulcular epithelium and that of the oral gingival epithelium. Within each of the treatment groups the paired t test was used to test the hypothesis that the mean difference was equal to zero. This was rejected ($P < 0.001$) in each of the three groups considered. The largest mean difference was observed in group 3 and the analysis of variance and Scheffe's method of multiple comparisons applied to the differences showed that group 3 differed significantly from both group 1 and group 2, but group 1 and group 2 were not significantly different from one another.

DISCUSSION

As shown in previous studies,[1, 2] all different combinations to control bacterial plaque tested were effective in producing keratinization of the sulcular epithelium.

Table 1
Mean Values, and Their Significance, for Different Modalities (ANOVA)

Treatment	Inflammatory index	Keratin length	Keratin width
1. Prophylaxis and antibiotics	9.48	3.11	0.19
2. Prophylaxis	14.43	2.86	0.25
3. Antibiotics	10.53	0.83	0.06
4. Control	25.06		

Indicates no significant difference (Scheffe's procedure).

Additionally, all of these reduced inflammation significantly when compared to the control. The differences observed in the mean inflammatory values for the three different treatment regimes were not high enough to show significance among them.

Although all different regimes controlled bacterial irritation enough to allow the epithelium to complete its full differentiation, the amount of keratin produced when only antibiotic therapy was used was significantly reduced, compared to the other two groups. This is true even when the Inflammatory Index obtained for group 3 (antibiotics only) was lower than that obtained in

Table 2
Comparison Between Keratin Width in Sulcular and Oral Gingival Epithelium (Paired t test)

Treatment	Keratin width		Difference	Significance
	Sulcular	Oral		
1. Prophylaxis and antibiotics	0.19	0.33	0.14	<0.001
2. Prophylaxis	0.25	0.35	0.10	<0.001
3. Antibiotics	0.06	0.28	0.22	<0.001

I Indicates no significant difference (Scheffe's procedure).

group 2 (prophylaxes only). As previously reported a relationship was observed between the width and the length of the keratin layer.[2]

As a consequence, these findings seem to indicate that the addition of the daily prophylaxes increased the epithelial response towards keratinization. The mechanical effect of the daily rubber cupping with a polishing paste in the gingival area might be responsible for these findings. It is worth stressing that the prophylaxes were performed aiming at cleaning the subgingival area as much as possible. These findings agree with the tendency towards parakeratinization reported after the evaluation of supervised intracrevicular toothbrushing techniques in humans.[4]

When the amount of keratinization obtained is compared with the width of keratin present in the oral gingival epithelium it is evident that the keratin layer is always less pronounced regardless of the treatment applied. However, this experiment was carried out only for 1 month; longer observations as in previous experiments might have differed in this respect.

From the results it seems safe to conclude that although antibiotics control inflammation within the tissues, mechanical stimulation is needed to increase the keratin within the sulcus. This is somewhat contrary to what has been reported previously when both therapeutic approaches were used in combination, in the sense that there seemed to be a correlation between the reduction of inflammation and the production of keratin.[1, 2] Consequently, inflammation needs to be controlled for the epithelium to exhibit its keratinizing potential, but the magnitude of this response may be well controlled by mechanical stimulation.

CONCLUSIONS

1. Systemic antibiotics, and local prophylaxes individually or combined, are effective in reducing inflammation within the gingival tissues.
2. The reduction in inflammation obtained by either method allows sulcular keratinization to develop.
3. Sulcular keratinization is significantly increased by performing daily subgingival prophylaxes.
4. Mechanical stimulation of the sulcular epithelium seems to promote its keratinization.

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